Kidney International, Vol. 59 (2001), pp. 1567–1573

Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy

Matthew R. Weir, Mary Traver Ward, Steven A. Blahut, David K. Klassen, Charles B. Cangro, Stephen T. Bartlett, and Jeffrey C. Fink

Division of Nephrology, Department of Medicine, and Division of Transplantation, Department of Surgery, University of Maryland School of Medicine, Baltimore, Maryland, USA

Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy.

Background. Chronic allograft nephropathy is the major cause of progressive renal failure in renal transplant recipients. It has no definitive treatment.

Methods. One hundred eighteen renal transplant recipients with declining kidney function and biopsy-proven chronic allograft nephropathy had their cyclosporine or tacrolimus dose reduced or discontinued with either the addition or continuation of mycophenolate mofetil and low-dose steroids at a mean of 853.3 days post-transplantation. Their renal function was modeled before and after this intervention by two methods: A least-square regression was used to assess the decay of renal function after the intervention and to compare that with the slope pre-intervention, whereas a hinge regression line method was used to assess the correlation of the intervention with the inflection point and the impact of the intervention on the decay of renal function. Mean follow-up was 651.0 days after the intervention. Serum creatinine at the time of intervention was 2.8 ± 0.9 mg/dL in the reduced dose cyclosporine ($N = 67$) and reduced dose tacrolimus ($N = 33$) groups, and was 2.7 ± 0.7 mg/dL in the group with discontinued calcineurin inhibitor ($N = 18$).

Results. Using the least-square method, 91.7% of the no calcineurin inhibitor group, 51.6% of the reduced dose cyclosporine group, and 59.3% of the reduced dose tacrolimus group had improved or lack of deterioration in slope after the intervention. Using the hinge regression line method, there was a statistically significant correlation of the inflection point with the intervention ($P = 0.001$). Moreover, there was a similar relationship with stabilized or improved graft function observed with the hinge regression line method and the least-square method, as 72.2% of the calcineurin inhibitor withdrawal group, 54.4% of reduced-dose cyclosporine group, and 40% of the reduced-dose tacrolimus group had improved the slope of decay of renal function or lack of deterioration after the inflection point. The difference between the calcineurin inhibitor withdrawal group and the reduced-dose cyclosporine/tacrolimus groups on the decay in renal function was significant ($P = 0.038$) with the least-square method and nearly significant ($P = 0.056$) using the hinge regression line method.

Conclusion. This intervention was safe, well tolerated, and associated with a minimal risk of acute rejection. We conclude that the reduction and possible withdrawal of calcineurin inhibitors may be necessary to slow the rate of loss of renal function in patients with chronic allograft nephropathy and deteriorating renal function.

Key words: progressive renal disease, transplantation, graft survival, hypertension, proteinuria, kidney graft, immunosuppression.

Received for publication August 8, 2000
and in revised form October 27, 2000
Accepted for publication November 2, 2000

Despite remarkable improvements in all forms of immunosuppression, allograft half-life has not improved as much as one-year graft survival rates [1, 2]. Thus, there may be an immunosuppression-resistant aspect of progressive kidney disease in the renal allograft recipient that results in a winnowing of renal function over time.

Chronic allograft nephropathy is a term defining progressive renal failure in renal transplant recipients. Clinically, it is characterized by a variable loss of renal function frequently associated with hypertension and proteinuria [3, 4]. Although numerous risk factors have been identified, its pathogenesis remains poorly defined. It develops more commonly in kidney grafts that have sustained either acute injury related to donor factors, preservation/injury issues or rejection, or to chronic injury, such as immunosuppressive drugs or hypertension, dyslipidemia, and carbohydrate intolerance [5–8].

Our center developed a policy in 1996 to employ a calcineurin inhibitor-sparing regimen in patients with declining kidney function caused by biopsy-proven chronic allograft nephropathy [9]. We have enrolled both cyclosporine (CsA)- and tacrolimus (FK)-treated transplant recipients. Their calcineurin inhibitor dose was reduced or completely discontinued. Immunosuppression support was maintained by either adding or continuing and/or increasing the dose of mycophenolate mofetil (MMF) and low-dose corticosteroids. As previously reported [9], our early observations demonstrating the short-term benefit on the rate of loss of renal function could be attributed,

in part, to a reduction of the nephrotoxic effects of the calcineurin inhibitors [10]. Although resolution of the functional aspects of the calcineurin inhibitor on glomerular filtration rate is helpful, improvement in histologic evidence of chronic allograft nephropathy may be more important for influencing graft half-life. This study reports our experience in over 100 patients with chronic allograft nephropathy with a more extended follow-up after calcineurin inhibitor reduction or cessation.

METHODS

Study design

Recipients of renal allografts with deteriorating renal function were identified and biopsied to assess the cause of the graft dysfunction. If patients had biopsy specimens that revealed chronic allograft nephropathy with minimal to no evidence of acute allograft rejection, they were enrolled in the clinical trial after giving written, informed consent. The immunosuppression regimen of these patients was then altered. The decision to reduce or eliminate the calcineurin inhibitor was arbitrary and at times was based on human lymphocyte antigen (HLA) matching and degree of renal insufficiency. Fewer patients were completely withdrawn because of concerns about risk for acute rejection. Patients receiving CsA had an approximate 50% reduction in their dose, resulting in a 12-hour trough level of approximately 50 to 125 ng/mL. Patients receiving FK also had an approximate 50% reduction in their levels, resulting in 12-hour trough levels of approximately 5 to 9 ng/mL. The decision to reduce or eliminate the calcineurin inhibitor was arbitrary and was based at time on HLA matching and degree of renal insufficiency. Fewer patients were completely withdrawn because of concerns about risk for acute rejection. Patients were either started on or had their dose of MMF adjusted to maintain 1 g orally twice a day, and low-dose corticosteroids (~0.1 mg/kg/day prednisone). Our primary goal was to compare the impact of this alteration in immunosuppression on the change in slope of renal function pre-intervention and postintervention in each patient and to assess the correlation between the timing of the intervention with the inflection point using a two-hinge regression line analysis. Our secondary analysis was to evaluate the impact of this intervention in the two different calcineurin inhibitor groups and in the group with complete calcineurin inhibitor discontinuation.

Immunosuppression

The majority of patients received induction therapy with either Minnesota antilymphocyte globulin (MALG, Minneapolis, MN, USA), antithymocyte globulin (ATGAM; Upjohn Company, Kalamazoo, MI, USA), or OKT3 (Orthoclone monoclonal antibody; Ortho-Biotech Pharmaceuticals, Raritan, NJ; USA). Once adequate graft function was established, patients were started on either CsA (Neoral, Sandoz Pharmaceuticals, Inc., East Hanover, NJ, USA) or FK (Prograf; Fujisawa Pharmaceuticals, Inc., Deerfield, IL, USA) usually within the first five- to seven-days post-transplantation. Each of the medications was adjusted in order to provide 12-hour trough levels of approximately 250 to 350 ng/mL of CsA and 10 to 14 ng/mL for FK. Steroids were started with 500 mg of intravenous methylprednisolone and rapidly tapered to 0.3 mg/kg/day by 30 days post-transplant. Patients were further tapered to approximately 0.1 mg/kg/day after the first year post-transplant. Some patients received azathioprine at an approximate dose of 2 mg/kg/day. This was discontinued at the study entry and mycophenolate mofetil (MMF; CellCept; Roche Pharmaceuticals, Nutley, NJ, USA) was started at 1 g orally twice a day. If patients received MMF from the time of transplantation, then the dose was adjusted to maintain 1 g twice a day as clinically tolerated.

Clinical care

Patients received standard clinical care as would be appropriate for any change in immunosuppression. Patients had chemistry and drug level monitoring weekly for four weeks, biweekly for two months, and then monthly through the follow-up period. Medication doses were adjusted as necessary for toxicity. Patients continued on routine antihypertensive medications to maintain a blood pressure of approximately 140/90 mm Hg or less. No patients received angiotensin-converting enzyme inhibitors or angiotensin type 1 receptor blockers. The majority of patients received calcium channel blockers, β blockers, α blockers, and diuretics as necessary to maintain appropriate blood volume. No effort was made to change dietary salt or protein intake.

Patient population

One hundred eighteen patients were enrolled in the study. Characteristics are shown in Table 1. One hundred patients had a reduction of their calcineurin inhibitor (CsA, $N = 67$; FK, $N = 33$). Eighteen patients had complete cessation of the calcineurin inhibitor. The decision to either reduce or withdraw completely the patients from calcineurin inhibitors was arbitrary. All patients were renal transplant recipients with the exception of one patient who also received a pancreas at the time of kidney transplantation.

Statistical methods

Changes in renal function were assessed using two different methods. The first method was the least-square (LS) linear slope of the P_{Cr}^{-1} versus time in months. β_0 was the intercept of the line and set to be the reciprocal of the lowest creatinine or nadir after day 30 for each

Table 1. Patient characteristics

	A Reduced CsA ($N = 67$)	B Reduced FK ($N = 33$)	C Reduced CsA and FK ($N = 100$)	D Discontinued CsA or FK ($N = 18$)	Statistical significance
Age *years*	43.7	47.3	44.9	49.5	
Male	43	24	67	14	
Female	24	9	33	4	
Caucasian	24	11	35	10	
African American	41	21	62	6	$P = 0.04$ Column C vs. D
Other	2	1	3	2	
CAD transplant	40	19	59	10	
LRD transplant	22	14	36	7	
Other/unknown transplant	5	0	5	1	
No antibody induction	10	11	21	6	$P = 0.03$ Column A vs. B
Delayed graft function	26	12	38	6	

Abbreviations are: CsA, cyclosporine; FK, tacrolimus; CAD, cadaveric; LRD, living related donor.

patient; β_1 was the linear slope derived for each patient. The second method was the hinged regression line based on the method described by Dunnigan, Hammen, and Harris [11]. The hinged regression line provided a β_1^0 and β_1^1 that were hinged at an inflection point between the first and the final observation times. The individual LS method slopes obtained for the individuals within each study group were compared using the Student's *t*-test for comparison of means. SPSS (Statistical Package for the Social Sciences; Chicago, IL, USA) and SAS (Statistical Analysis Software, Cary, NC, USA) were the software packages used for the analysis.

RESULTS

Patient and donor characteristics

As depicted in Table 1, the study population consisted predominantly of men (68.6%), African Americans (57.6%), and recipients of cadaveric renal transplants (58.5%). There were no major differences between the reduced and discontinued calcineurin inhibitor groups except for race and gender. There were no differences in HLA matching between these two groups (mean match for the whole cohort).

The mean nadir creatinine post-transplantation for the entire group was 1.7 ± 0.6 mg/dL (range 0.8 to 4.3 mg/dL). Figure 1 illustrates a box plot of best nadir serum creatinines in the participants of each group prior to the intervention. Pre-intervention incidence of acute rejection was lower ($P = 0.01$) in the no calcineurin inhibitor group. Despite this, there was no difference in serum creatinine at the time of intervention in the combined CsA and FK reduction groups (2.8 ± 0.9 mg/dL) and the group with discontinued calcineurin inhibitor (2.7 ± 0.7 mg/dL; Table 2). The mean intervention time post-transplantation was 853.3 days.

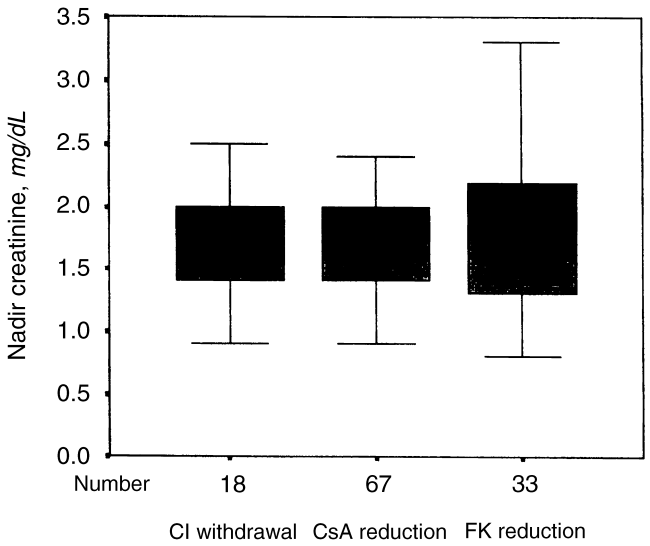

Fig. 1. Best nadir of serum creatinine (mg/dL levels in study participants prior to the intervention. Abbreviations are: CI, calcineurin inhibitor; CsA, cyclosporine; FK, tacrolimus.

The mean follow-up time was 651.0 days after the intervention. CsA-treated patients had their intervention later post-transplantation than FK patients ($P = 0.0004$). The duration of follow-up was longer in the CsA reduction group ($P < 0.001$), which may explain the greater long-term reduction in CsA levels ($P < 0.001$) than in the FK reduction group, as we gained confidence in the safety of progressive calcineurin inhibitor reduction. MMF was started at the intervention point in 37 (of 67) CsA reduction patients and 0 (of 33) FK reduction patients, and in 9 (of 18) calcineurin inhibition with-

Table 2. Renal function and drug levels

	A Reduced CsA ($N = 67$)	B Reduced FK ($N = 33$)	C Reduced CsA and FK ($N = 100$)	D Discontinue CsA or FK ($N = 18$)	Statistical significance
Nadir creatinine *ng/dL*	1.7	1.8	1.7	1.8	
Creatinine at intervention *mg/dL*	2.8	2.7	2.8	2.7	
Pre-intervention acute rejection	47	25	72	7	$P = 0.01$, Columns A, B and C vs. D
Pre-intervention time post-TX *days*	635	1154	806	463	
Post-intervention time *days*	853	478	729	792	$P < 0.001$, Column B vs. A Column B vs. D
Cyclosporine level pre-intervention *12-hour trough, ng/dL*	235.0	X	X	264.6	
Cyclosporine level post-intervention *12-hour trough, ng/dL*	132.1	X	X	0	$P < 0.001$, CsA level pre vs. post
Tacrolimus level pre-intervention *12-hour trough, ng/dL*	X	12.1	X	X	
Tacrolimus level post-intervention *12-hour trough, ng/dL*	X	9.7	X	0	$P =$ NS, FK level pre vs. post
MMF *daily dose/mg*	1234.7	1577.5	1361.4	1265.6	$P < 0.001$, Column B vs. A, $P < 0.02$, Column B vs. D
Started on MMF at intervention	37	0	67	9	

Abbreviations are: TX, transplantation; MMF, mycophenolate mofetil.

Table 3. Clinical characteristics pre- and post-intervention

	Pre-intervention		Post-intervention	
	Reduced CsA/FK	CI withdrawal	Reduced Csa/FK	CI withdrawal
Systolic BP *mm Hg*	143.2 ± 8.3	140.0 ± 2.7	137.7 ± 0.9	139.5 ± 2.0
Diastolic BP *mm Hg*	81.6 ± 0.4	84.7 ± 2.0	81.1 ± 0.5	82.0 ± 1.6
Serum glucose *mg/dL*	137.5 ± 2.0	140.0 ± 5.7	137.0 ± 2.5	117.4 ± 4.7[a]
Serum cholesterol *mg/dL*	210.6 ± 1.3	232.4 ± 6.7	201.0 ± 1.6	194.7 ± 3.6[a]

Abbreviations are: BP, blood pressure; CsA, cyclosporine; FK, tacrolimus; CI, calcineurin inhibitor. Data are expressed as mean ± SEM.
[a] $P < 0.05$

drawal patients, and was continued in the remainder of patients. The mean daily MMF dose was statistically higher in the FK reduction group, perhaps because of the shorter duration of follow-up. There were no differences in mean MMF dose (mg per day) in those patients on the drug preintervention compared with postintervention (data not shown).

Table 3 illustrates important clinical characteristics pre-intervention and postintervention that could affect the rate of loss of renal function. Note that the systolic blood pressure decreased in the combined reduced dose groups from 143.2 to 137.7 mm Hg (P = NS), whereas there was little change in diastolic pressure. There was minimal change in blood pressure, systolic or diastolic, in the group with complete calcineurin inhibitor withdrawal. However, this group had the greatest incidence of improvement of renal function. This suggests that, in part, the benefit on renal function with the withdrawal of calcineurin inhibitors is not related to an improvement in blood pressure, although this observation may not hold with a longer follow-up.

However, in the complete withdrawal group, there was a statistically significant improvement in mean serum cholesterol (232.4 to 194.7 mg/dL, $P = 0.00$) and in mean serum glucose (140.0 to 117.4 mg/dL, $P = 0.003$), indicating in part that improvement in metabolic parameters may be important in the improved stabilization of renal function seen in these patients.

When evaluating the reduced dose groups of CsA or FK individually, there were no differences in serum cre-

atinine before and after the intervention. There were no changes in serum cholesterol or random glucoses after dose reduction either within or between groups. Systolic and diastolic blood pressure measurements did not change significantly after CsA reduction (134.6/81.0 to 135.9/80.3 mm Hg). In the FK group after a dose reduction, there was a significant reduction of systolic blood pressure (170.4/83.5 to 143.3/83.6 mm Hg, $P < 0.05$).

Safety and tolerability

Thirty-three patients had 61 biopsies during the follow-up period to evaluate increases in serum creatinine. Nine patients on 2 g MMF/day had biopsies indicating minimal or borderline rejection. All received additional steroid therapy. No patients lost their graft. Only one patient needed retreatment with steroids. Two patients on no calcineurin inhibitors had questionable mild and type 1A rejection; both received steroids and stabilized. Four patients on reduced FK and 500 to 1000 mg MMF per day had biopsies (3 mild, 1 type 1A). All received steroids and were stabilized. Three patients were noncompliant and had biopsies (mild, minimal, type IIB) indicating acute rejection. One lost their graft.

Mycophenolate mofetil was well tolerated. All patients were able to maintain at least a dose of 500 mg twice a day. The majority received a dose of 1.5 g per day (Table 2). Dosage adjustment was necessary in some patients based on the presence of anemia and gastrointestinal intolerance. No patient required a reintroduction of calcineurin inhibitors or escalation of dose.

Analysis of intervention on renal slopes

Renal function was modeled before and after the intervention by employing a LS and two-hinge regression line method. The two-hinge regression line method was also used to assess the correlation of the intervention with the actual change in slope of renal function.

Using the LS method, 91.7% of the calcineurin inhibitor withdrawal group, 51.7% of the reduced dose CsA group, and 59.3% of the reduced dose FK group had improved or lack of deterioration in slope after the intervention (Fig. 2). Similarly, using the hinge regression line method 72.2% of the calcineurin inhibitor withdrawal group, 54.4% of the reduced dose CsA group, and 40% of the reduced dose FK group had improved slope of decay of renal function and lack of deterioration after the inflection point. The difference between the calcineurin inhibitor withdrawal group and the reduced dose CsA/FK groups on the decay of renal function was significant with the LS method ($P = 0.038$) and was nearly significant using the hinge regression line method ($P = 0.056$). There was no difference between either of the reduced dose (CsA or FK) groups on the incidence of stabilization or improvement in renal function postintervention despite the striking visual differences in Figure 2. This lack of difference was caused by the inherent variability in the decay of renal function observed in each of the patients.

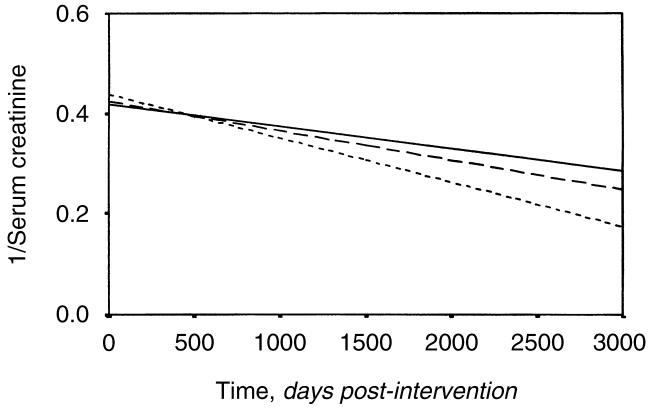

Fig. 2. Slopes showing loss of renal function over time using the least square mean. Symbols are: (solid line) no calcineurin inhibitor; (dashed line) cyclosporine; (dotted line) tacrolimus.

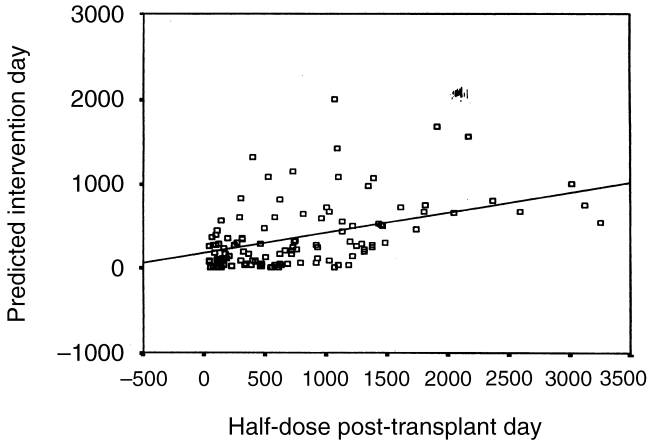

Fig. 3. Correlation of the inflection point of the hinge regression analysis with the actual intervention (reduction or withdrawal of the calcineurin inhibitor; $r^2 = 0.23$; $P = 0$).

The hinge regression line method demonstrated a significant correlation between the intervention (either calcineurin inhibitor reduction or withdrawal) and change in slope after the inflection point (Fig. 3), indicating that the improvement in renal function was not a random event.

DISCUSSION

Chronic allograft nephropathy is the second most important cause of graft loss post-transplantation after death with functioning graft [12]. It is the most prevalent cause of progressive graft failure in the first decade post-transplant [13]. Despite the overwhelming importance of this multifactorial disease entity, there is little understanding about how to intervene therapeutically.

Much concern remains with regard to the relationship of immunologic issues [14, 15], nephron dosing [16], blood pressure [17], metabolic factors [5, 7, 8], and progression of disease. However, also of concern is the therapeutic benefit of the calcineurin inhibitors, CsA and FK, in patients with declining kidney function caused by chronic allograft nephropathy [6, 18–20]. The relative contribution of these drugs to this disease process, once initiated, remains unknown. Certainly, their prophylactic ability against rejection is extremely important. However, after renal function starts to deteriorate with histologic evidence of fibrosis and vascular hyalinosis, are these drugs beneficial or detrimental?

The results of our ongoing clinical trial suggests that a relationship exists between reduced calcineurin inhibitor dosing or withdrawal and stabilization of renal function. Moreover, there did not appear to be any significant risk of provoking acute rejection with late reduction (more than two years post-transplant) in the doses of the calcineurin inhibitors by using MMF, especially 1 g twice a day, and low-dose corticosteroids.

Since chronic allograft nephropathy is a heterogenous process with a variable rate of loss of kidney function, modeling the decay of kidney function has proven to be difficult [21]. Our own experience in comparing LS versus hinge regression line analyses to predict decay of renal function in transplant recipients indicates a better predictability with the hinge regression line method (abstract; Fink et al, *J Am Soc Nephrol* 10:728A 1999). To be thorough, we employed both methods to analyze our data. There was a general consistency in the observations with both methods, indicating that approximately 50% of the patients with reduced dose calcineurin inhibitors (either CsA or FK) had an improved slope of decay in renal function or lack of deterioration after the intervention. Moreover, complete withdrawal of the calcineurin inhibitors, regardless of the statistical method employed to model renal function, demonstrated a statistically significant improvement in the rate of loss of renal function. The hinge regression line method also conclusively demonstrated the significant relationship between the intervention and the change in slope of renal function.

The shortcomings of our analysis revolve around the many issues of post-transplantation care, such as determinations of blood pressure, glycemia, and cholesterol. No organized efforts were made to control for these factors, other than to reduce blood pressure to traditionally accepted levels (<140/90 mm Hg) and to treat symptomatic diabetes mellitus or abnormal elevations of cholesterol with statins. The subtle improvement in systolic blood pressure in the reduced CsA/FK groups may have played a role in the improvement observed. However, the complete withdrawal group, which derived the greatest benefit, had no significant change in blood pressure, indicating that other factors may be important. The complete withdrawal group did have a significant reduction in both random serum glucose and cholesterol measurements. It is possible that improved metabolic parameters did play a role in the stabilization of renal function in these patients.

Similarly, possible differences in donor factors within each group could contribute to differences seen in our analysis. However, since there was not a difference in nadir serum creatinine between the groups, nor was there a difference in slopes after the intervention between living related (-0.003 ± 0.003 mg/dL^{-1}) or cadaveric (-0.0003 ± 0.0002 mg/dL^{-1}) recipients, the likelihood of donor differences having an impact on outcome is less likely.

Our results indicate that reduction or withdrawal of calcineurin inhibitors does not help all patients. There are clearly other factors that contribute to graft deterioration. The interplay between immunologic and nonimmunologic factors remains an area of substantial debate. Moreover, there is scientific interest in whether graft injury caused by ischemia, diabetes, hypertension, or rejection, in and of itself, contributes to immunologic injury via either direct or indirect alloantigen presentation pathways [22, 23].

The mechanism behind the benefit of calcineurin inhibitor reduction or withdrawal is unknown. Experimental data indicate that CsA may promote development of glomerular and tubulointerstitial injury by stimulation of profibrogenic cytokines such as transforming growth factor-β [24–27]. These cytokines promote excessive deposition of extracellular matrix proteins and may even interfere with their degradation [28, 29]. There are also experimental data suggesting that CsA induces apoptosis leading to the loss of supporting extracellular matrix architecture, which could interfere with the maintenance of proper tissue organization [30]. Although not as well studied, there are concerns that FK may induce similar problems.

We have previously reported that after one year of CsA reduction in patients with chronic allograft nephropathy, there is diminished immunohistochemical staining for angiotensin II, angiotensin type 1 receptor, and transforming growth factor-β1 in sequential kidney biopsy specimens (abstracts; Wei et al, *J Am Soc Nephrol* 10:92A, 1999, and Song et al, *Transplantation* 67:S236, 1999). These changes correlate with an improvement in the histologic observations of chronic allograft nephropathy with decreased tubular atrophy, vascular hylanosis, and interstitial fibrosis. Moreover, we have similarly demonstrated diminished apoptotic events in sequential kidney biopsy specimens of patients with chronic allograft nephropathy after CsA level reduction (abstract; Wei et al, *Transplantation* 67:S51, 1999). Whether these changes are a direct or indirect response to calcineurin inhibitor reduction is unknown. Also unknown is whether there

are possible differences between the two calcineurin inhibitors, CsA and FK, in their propensity for inducing these changes or whether the possibility of regression exists once the drug exposure is reduced. Our current evaluation did not evidence any differences in effects of the two drugs after dose reduction on changes in slopes of renal function. However, the dose of CsA was reduced more significantly than the FK.

We conclude from our experience that a reduction and possible cessation of calcineurin inhibitors may be necessary to slow the rate of loss of renal function in patients with biopsy-proven chronic allograft nephropathy and deteriorating renal function. Although this intervention is safe, well tolerated and associated with a minimal incidence of acute rejection, it is clearly not the only strategy that will need to be undertaken in order to prolong graft half-life. We would recommend that this strategy be considered as part of a multidisciplinary approach, including intensive control of blood pressure, glycemia, and cholesterol metabolism.

We also believe that more clinical trials are needed to develop optimal calcineurin inhibitor-sparing regimens in patients with graft dysfunction. With the advent of new induction regimens such as anti-CD 25 monoclonal antibodies and non-nephrotoxic immunosuppressants like sirolimus and MMF, more opportunities to prolong graft life exist than ever before.

ACKNOWLEDGMENTS

We wish to acknowledge the expert secretarial assistance of Vondalee Cowling and an unrestricted educational grant from Roche Pharmaceuticals, Inc.

Reprint requests to Matthew R. Weir, M.D., Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, 22 South Greene St., Baltimore, Maryland 21201-1595, USA.
E-mail: mweir@medicine.umaryland.edu

REFERENCES

1. Cecka J: The UNOS scientific transplant registry: Ten years of kidney transplants. *Clin Transplant* 4:1–14, 1997
2. Hariharan S, Johnson CP, Bresnahan BA, *et al:* Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 342:605–612, 2000
3. Massy ZA, Guijarra C, Wiederkehr MR, *et al:* Chronic renal allograft rejection: Immunologic and nonimmunologic risk factors. *Kidney Int* 48(Suppl 52):S107–S111, 1995
4. Paul LC: Chronic allograft nephropathy: An update. *Kidney Int* 3:783–793, 1999
5. Almond PS, Matas A, Gillingham K, *et al:* Risk factors for chronic rejection in renal allograft recipients. *Transplantation* 55: 752–757, 1993
6. Olyaei AJ, de Mattos AM, Bennett WM: Immunosuppressant-induced nephropathy. *Drug Safety* 21:417–488, 1999
7. Halloran PF, Melk A: Rethinking chronic allograft nephropathy: The concept of accelerated senescence. *J Am Soc Nephrol* 10:167–181, 1999
8. Hamar P, Muller V, Kohnle M, *et al:* Metabolic factors have a major impact on kidney allograft survival. *Transplantation* 64:1135–1139, 1997
9. Weir MR, Anderson L, Fink JC, *et al:* A novel approach to the treatment of chronic allograft nephropathy. *Transplantation* 64:1706–1710, 1997
10. Weir MR, Fink JC, Hanes DS, *et al:* Chronic allograft nephropathy: Effect of cyclosporine reduction and addition of mycophenolate mofetil on progression of renal disease. *Transplant Proc* 31: 1286–1287, 1999
11. Dunnigan GM, Hammen JL, Harris TR: A SAS-IML program for implementing two-phase regression analysis of geophysical time series data. *Comput Geosci* 23:763–770, 1997
12. Matas A, Gillingham K, Sutherland D: Half-life and risk factors for kidney transplant outcome-importance of death with function. *Transplantation* 55:757–761, 1993
13. Schweitzer EJ, Matas AJ, Gillingham KJ, *et al:* Causes of renal allograft loss: Progress in the 1980s, challenges for the 1990s. *Ann Surg* 214:679–688, 1991
14. Matas A, Gillingham K, Payne W, *et al:* The impact of an acute rejection episode on long-term renal allograft survival (t1/2). *Transplantation* 57:857–859, 1994
15. Lindholm A, Ohlman S, Albrechtsen D, *et al:* The impact of acute rejection episodes on long-term graft function and outcome in 1347 primary renal transplants. *Transplantation* 56:307–315, 1993
16. McKenzie HS, Tullius SG, Heemann UW, *et al:* Nephron supply is a major determinant of long-term renal allograft outcome in rats. *J Clin Invest* 94:2148–2152, 1994
17. Opelz G, Wujciak T, Ritz E, for the Collaborative Transplant Study: Association of chronic kidney graft failure with recipient blood pressure. *Kidney Int* 53:217–222, 1998
18. Pankewycz OG, Miao L, Issacs R, *et al:* Increased renal tubular expression of transforming growth factor beta in human allografts correlates with cyclosporine toxicity. *Kidney Int* 50:1634–1640, 1996
19. Benigni A, Bruzzi I, Mister M, *et al:* Nature and mediators of renal lesions in kidney transplant recipients given cyclosporine for more than one year. *Kidney Int* 55:674–685, 1999
20. Solez K, Vincenti F, Filo RS: Histopathologic findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tacrolimus versus cyclosporine: A report of the FK506 Kidney Transplant Study Group. *Transplantation* 66:1736–1740, 1998
21. Kasiske BL, Heim-Duthoy KL, Tortorice KL, Rao KV: The variable nature of chronic declines in renal allograft function. *Transplantation* 51:330–334, 1991
22. Tullius SG, Tilney NL: Both alloantigen-dependent and independent factors influence chronic allograft rejection. *Transplantation* 59:313–318, 1995
23. Womer LL, Vella JP, Sayegh MH: Chronic allograft dysfunction: Mechanisms and new approaches to therapy. *Semin Nephrol* 20: 126–147, 2000
24. Shihab FS, Andoh TF, Tanner AM, *et al:* Role of transforming growth factor-β1 in experimental chronic cyclosporine nephropathy. *Kidney Int* 49:1141–1151, 1996
25. Sharma VK, Bologa RM, Xu G-P, *et al:* Intragraft TGF-β1 mRNA: A correlate of interstitial fibrosis and chronic allograft nephropathy. *Kidney Int* 49:1297–1303, 1996
26. Nicholson ML, Bicknell GR, Barker G, *et al:* Intragraft expression of transforming growth factor β1 in isolated glomeruli from human renal transplants. *Br J Surg* 86:1144–1148, 1999
27. Cuhaci B, Kumar MSA, Bloom RD, *et al:* Transforming growth factor β levels in human allografts chronic fibrosis correlate with rate of decline in renal function. *Transplantation* 68:785–790, 1999
28. Border WA, Noble NA: Transforming growth factor-beta in tissue fibrosis. *N Engl J Med* 331:1286–1292, 1994
29. Shihab FS, Yamamoto T, Nast CC, *et al:* Transforming growth factor-β and matrix protein expression on acute and renal chronic rejection of human renal allografts. *J Am Soc Nephrol* 6:286–294, 1995
30. Shihab FS, Andoh TF, Tanner AM, *et al:* Expression of apoptosis regulatory genes in chronic nephrotoxicity favors apoptosis. *Kidney Int* 56:2147–2159, 1999